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# Toxicity and sub-lethal effect of endemic plants from family Anacardiaceae on oviposition behavior of *Aedes albopictus*

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ABSTRACT

**Objective:** To evaluate the lethal concentration, oviposition deterrence and ovicidal activity of acetone extracts of *Melanochyla fasciculiflora* (*M. fasciculiflora*) leaf and *Gluta renghas* (*G. renghas*) leaf against *Aedes albopictus* (*Ae. albopictus*).

**Methods:** To determine the lethal concentration of Anacardiaceae, ten test concentrations of the extracts ranging from 200 to 650 mg/L were selected for larvicidal bioassays and 25 early fourth instar larvae were exposed to the extracts for 24 h. The sub-lethal concentrations used for oviposition deterrence was the value of $LC_{25}$, $LC_{50}$ and $LC_{75}$ from above study which is 235 mg/L, 470 mg/L and 705 mg/L for *M. fasciculiflora* extract and 187.5 mg/L, 375 mg/L and 562.5 mg/L for *G. renghas* extract, respectively. Twenty gravid *Ae. albopictus* were allowed to oviposit in different treated concentrations. For oviciding procedure, a total of 300 eggs of *Ae. albopictus* were soaked in solution with each treated concentration as mentioned above for 24 h. After 24 h, eggs were sieved and soaked in seasoned water, and hatching rates were calculated. For comparison, only seasoned water was used in control experiment.

**Results:** *G. renghas* demonstrated lower $LC_{50}$ value of 372.80 mg/L compared to *M. fasciculiflora* (467.90 mg/L). The activity index of negative oviposition revealed the deterrent effect and thus, caused a remarkable negative response resulting in oviposition of fewer eggs compared with control (without plant extract). The acetone extract of *M. fasciculiflora* was more effective than *G. renghas* extract in displaying oviposition deterrence potential since the latter did not possess the deterring effect within the concentration range tested. However, both plant extracts exhibited excellent oviciding effect as 92.33% of eggs failed to be hatched when treated with 705.0 mg/L of *M. fasciculiflora* and 86.67% with 562.5 mg/L of *G. renghas*. The oviposition deterrence and percentage of egg mortality were directly proportional to the concentrations of extracts in both plants tested.

**Conclusions:** These results clearly indicate that the acetone extract of *G. renghas* could be served as potential larvicide, whereas *M. fasciculiflora* has better sub-lethal effect for oviposition deterrence and against *Ae. albopictus* as an oviciding agent.

## 1. Introduction

To date, *Aedes albopictus* (*Ae. albopictus*) is capable of transmitting 26 arboviruses, which comprise of genus *Flavivirus*, genus *Alphavirus*, genus *Orbivirus*, genus *Picornavirus*, genus *Bunyavirus* and genus *Phlebovirus* [1]. Most of the vector controls in the world are assisted by synthetic chemical insecticides since insecticides are the cheapest, easiest and most rapidly effective in control approach. However, when the effectiveness of residual house-spraying dichlorodiphenyltrichloroethane was greatly reduced in controlling mosquito populations, scientists began to realize the problem of resistance. As of now, the resistance problem (more than 100 mosquito species were reported as resistant to one or more insecticides) still persists, and even the vector control programs have been

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switched to use of other insecticides such as pyrethroids, organophosphates, and carbamates [2]. During the past decades, the main factor driving the widespread resistance is the heavy reliance on a single class of insecticides, the pyrethroids [3].

Due to insistence of insecticide resistance problem, the new control agent obtained from natural products has played a vital role in controlling insect vector. Botanical insecticides or so-called naturally occurring insecticides such as neem, rotenone and so forth have been proven to be effective in vector control. Botanical insecticides are relatively safe, rapidly effective, decomposable, and readily available sources which can be obtained from the nature [4]. Due to these factors, many researches are investigated by utilizing the plant extraction as the approach to control mosquitoes for advance use. Plants have been studied due to biochemical properties as an alternative agent for pest control because plant can produce diverse organic chemical compounds regardless of involvement in growth and development of plants, and these compounds are simplistically called secondary metabolites [5,6]. The most effective way to deal with the mosquitoes is to use their biological behaviors as a weapon to control the mosquito population. It is obviously clear that distribution of oviposition sites is relevant to disease propagation [7,8]. When the process of oviposition is inhibited, the population of mosquitoes will be reduced and thereby the probability of getting infected with mosquito-borne disease will be reduced. Hence, nowadays, research on oviposition has become the focus in the concept of integrated vector control management [9,10].

The selected plants chosen to be used in this study are commonly known as Renghas in Malaysia for both species of *Gluta renghas* (*G. renghas*) and *Melanochyla fasciculiflora* (*M. fasciculiflora*) [11]. Both species belong to family Anacardiaceae; with endemic taxa, this family are naturally occurring and widely distributed in Peninsular Malaysia and Borneo [12]. Rengas trees can be very hazardous because they can secrete noxious substances which will cause dermatitis [13]. The detrimental substances in *Gluta* spp. can be found in the fruits, leaves, roots, sap and even timbers [14]. These substances are usually made up of mono- or di-hydric phenols or monohydric phenolic [15]. Most of the variety of phenols is originated from phenylalanine, tyrosine or tryptophan which is one of the secondary metabolites [16,17]. Hence, the noxious substance may prove that the plants in family Anacardiaceae can possibly become one of the biopesticides due to the organic chemicals or secondary metabolites. Apart from these, both species are endemic in Malaysia, hence the source is available and ready to prepare.

Therefore, our study aimed to find the lethal concentration from two endemic species of plants found in Malaysia, namely, *M. fasciculiflora* and *G. renghas* as one of the sources of biopesticides. We also investigated the sub-lethal effects for oviposition deterrence and ovicidal activity against *Aedes* (*Stegomyia*) *albopictus* (Skuse). To the best of our knowledge, no information is available on the efficacy of these two plants.

## 2. Materials and methods

### 2.1. Wild strain of mosquito colonies

*Ae. albopictus* wild strain was collected from secondary forest located at main campus area of Universiti Sains Malaysia (5°21′ N, 100°18′ E) by using ovitrap method. Ovitraps with wooden paddle were placed in the area which will not be disturbed by children or pets, away from excess rainwater, close to the accumulated trash or any expected breeding sites and where direct sunlight is avoided. The wild strain was collected after four days. Collected larvae and paddles were brought back to laboratory and the larvae were reared until adults. The eggs on paddles were immersed in an enamel tray containing chlorine-free water. Hatching occurred within 24 h. The larval food made of dog biscuit, bovine liver, yeast and milk powder at the ratio of 2:1:1:1 was given at 1 mg daily. After pupation, the pupae were transferred and placed in the standard mosquito rearing cage (30 cm × 30 cm × 30 cm). Once the adults emerged, cotton soaked with 10% sucrose solution with B-complex was provided continuously before blood feeding and they were allowed to mate. After two to five days, female adults were then offered with blood feeding of white mouse confined in a wire. After fed with blood, the mosquitoes were allowed to rest for 2 days for the development of eggs before the experiment was carried out. The culture and all experiment were maintained and ran at (28 ± 2) °C, 70%–85% relative humidity with a photo period of 14 h light: 10 h dark.

### 2.2. Extraction of plant leaves

Two species of plants from family Anacardiaceae were chosen for this study. *G. renghas* and *M. fasciculiflora*, both of which are endemic plants in Malaysia, were chosen. Both plant leaves were collected from Penang National Park, Penang (5°27′ N, 100°12′ E). The leaves were rinsed with tap water and shade dried at the normal environment temperature. The dried leaves were powdered mechanically by using commercial electrical stainless steel blender. The powdered plant leaves were extracted with acetone solvent by using the Soxhlet apparatus. A total of 40 g of powdered plant leaves were inserted into paper thimble (43 mm × 123 mm) and mixed with pebbles in order to ensure that optimum solvent flows through the plant powder. The thimble was closed with cotton wool and extraction was started by using Soxhlet apparatus. The boiling point of acetone was set at 50.5 °C. The apparatus ran for 3 to 4 cycles and the procedure was repeated twice by replacing the plant powder for each round in the paper thimble. The extract yield then went through evaporation process by using rotary evaporator under 80 to 100 r/min at 60 °C to evaporate the excess acetone solvent. The crude extract was stored in oven at 37 °C for further drying process.

### 2.3. Mosquito larvicidal bioassay

To prepare stock solution, 1 g of crude extract was weighed and dissolved in 100 mL of acetone to produce 10 000 mg/L of stock solution [18]. Larvicidal bioassays were performed as per standard of World Health Organization larval susceptibility test method [19]. Bioassay was performed in 350 mL paper cups containing 250 mL of test medium (distilled water and plant extract solution) and 25 *Ae. albopictus* of early fourth instar larvae were exposed to it for 24 h. A homogenous population of late third instar larvae (5 days old and 4–5 mm in length) were chosen for this study [19]. Initially, the mosquito larvae were exposed to a wide range of test concentrations to find out the activity range of the extract solutions. Ten concentrations ranging from 200 to 650 mg/L yielding between 0% and 100% mortality in 24 h of exposure were selected for larvicidal bioassays. A total of three